World Journal of Dairy & Food Sciences 7 (2): 167-173, 2012
ISSN 1817-308X

DOI: 10.5829/idosi.wjdfs.2012.7.2.1110

Antihyperglycemic Effect of Chicory Leaves and Vanadium Consumption on Diabetic Experimental Rats

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Abstract: This study was carried out to investigate the effect of cichorium leaves and vanadium consumption on diabetic rats. Forty two Sprague Dawley adult male rats were injected with a single intraperitoneal dose of streptozotocin to induce diabetes then classified into control positive group (C+) and five treated groups which were Amaryl drug group (AD), cichorium powder group (CP), cichorium extract group (CE), cichorium powder with vanadium group (CPV) and cichorium extract with vanadium group (CEV). All the treated groups showed significant increase in weight gain and feed efficiency ratio and the more improvement in nutritional results appeared in CEV. Values of insulin, hemoglobin, glutathione-peroxidase, superoxide dismutase and catalase in serum and glycogen in liver were significantly increased in all treated groups compared with C+ group. Values of serum alanine and aspartate aminotransferase enzymes (ALT and AST), glucose and glucosalated heamoglobin were significantly decreased in all treated groups. Also, values of cholesterol, triglyceride, low density lipoprotein cholesterol and nitric oxide in serum, cholesterol and total lipid in liver and atherogenic index were significantly decreased in all treated groups compared with C+ group. Serum alkaline phosphatase and creatinine were significantly decreased but high density lipoprotein cholesterol was significantly increased in CP, CE, CPV and CEV groups but serum urea was insignificantly difference compared with C+ group. This study recommended that the intake of cichorium extract with vanadium may be useful for treating diabetes.

Key words: Chicory · Vanadium · Antioxidant enzymes · Diabetes mellitus · Rats

INTRODUCTION

Diabetes mellitus is a major endocrine disorder, affecting nearly 10% of the population all over the world. Diabetes mellitus is a complex disorder that disturbs the metabolism of carbohydrates, fat and protein. Diabetes mellitus characterized by metabolic disorders related to high levels of serum glucose [1]. Diabetes is also one of the leading causes of kidney failure, whereas heart disease accounts for the majority of deaths among people with diabetes in developed countries. Medicinal plants play an important role in the management of diabetes mellitus especially in developing countries [2]. Chicory *(Cichorium intybus* L.*)* is a root vegetable and a perennial plant with blue or white flowers. Green leafy part of chicory is often used in cooking or in salads. It is perhaps best known for the roasted roots used as the traditional coffee substitute with no caffeine and less well known as grazed forage for ruminants. It has long been used as renal protective and anti-inflammatory [3, 4]. It has been prescribed in various forms for the treatment of gastrointestinal disorders including gastric ulcers. Chicory roots or leaves extracts revealed that they produce hepatoprotective, antihyperglycemic and antioxidant effects [5-7]. Clinical research has confirmed the efficacy of several plant extracts in the modulation of oxidative stress associated with diabetes mellitus b. The aqueous extract of chicory leaves has been shown to have a marked radical scavenging properties and offered significant protection against protein oxidation and DNA damage, which could be attributed to the presence of phenolic compounds [5, 8]. Vanadium is essential micronutrient. High doses of vanadium have been tested as an aid to controlling blood sugar levels in people with diabetes [9]. Vanadium may act as a co-factor for enzymes involved in blood sugar metabolism, lipid and cholesterol metabolism, bone and tooth development, fertility, thyroid function, hormone production and neurotransmitter metabolism [10]. Vanadyl sulfate is one of the element vanadium's colorful forms and it is sometimes called a

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vanadium salt. The insulin-like properties of vanadium have been attributed to the cationic form, vanadyl, into which the anionic form, vanadate, is reduced within cells. So, vanadyl sulfate is the form of choice for the treatment. Vanadyl sulfate treatment of diabetes is related to their antihyperglycemic, antihyperlipemic and hyperinsulinemic effects [11, 12].

This study was designed to study the effect of combination of chicory (plant source) and vanadium (mineral source) on diabetic experimental rats.

MATERIALS AND METHODS

Materials: Streptozotocin was procured from Sigma, St. Louis, MO, USA. Vanadyl sulfate-3-hydrate was obtained from Hanawa Extra Pure Reagent China. Antidiabetic drug (Amaryl) was produced by Saofi-Avents Egypt under license of Saofi-Avents, Germany. Biochemical kits were purchased from Alkan Co. for Chemicals and Biodignostics, Dokki, Egypt. Chicory dry leaves (*Cichorium intybus*, Family Asteraceae) were obtained from the local market of herbs and medicinal plants, Dakahleia Governorate, Egypt and scientifically identified at Horticultural Research Institute, Agricultural Research Center, Egypt. A total of forty-two Sprague-Dawley adult male rats were purchased from the National Research Center, Giza, Egypt and weighted 109±5 g. The standard diet was performed according to NRC [13].

Methods: The dried leaves of chicory were crushed to fine powder. Water extract of cichorium was prepared by adding cichorium to distilled water (1:5 wt/v) and mixing for 10 min at 100°C then filtrating. Hand refractometer was used to measure the concentration by determining the refractive index of solutions that were adjusted to 5 % for each herb in the final solution [14]. Rats were fed on standard diet for a week as adaptation period. Food and water were provided *ad-libitum*. The rats were injected with a single intraperitoneal dose of streptozotocin (55 mg/kg body weight) in 0.1 M citrate buffer of pH 4.5 then supplied with 5% glucose solution for 48 h after injection in order to prevent hypoglycemia [15]. After four days, blood samples were taken from orbital plexus for estimation of glucose. The rats having persistent hyperglycemia were considered as diabetic rats and used for the experiment. The diabetic rats were classified into the following groups:

- Control positive group (C+): that provided standard diet without treatment.
- Amaryl group (AD): that provided standard diet with 0.36 mg/Kg body weight of Amaryl drug dissolved in distilled water and given to rats by oral intubations.
- Cichorium powder group (CP): that provided standard diet with 15% of cichorium powder in diet.
- Cichorium extract group (CE): that provided standard diet with 1 ml of 5% of cichorium extract and given to rats by oral intubations.
- Cichorium powder with vanadium group (CPV): that provided standard diet with 15% of cichorium powder in diet and 10 mg/Kg body weight of vanadium that dissolved in distilled water and given to rats by oral intubations.
- Cichorium extract with vanadium group (CEV): that provided standard diet with 1 ml of cichorium extract and 10 mg/Kg body weight of vanadium that dissolved in distilled water given to rats by oral intubations.

Daily food intake and weekly body weight gain were recorded. Feed efficiency ratio (FER) was determined by Chapman *et al.* [16]. At the end of experiment (60 days), rats were anesthetized, blood sample were collected. The liver of sacrificing rats was collected separately and perfuse with 50 to 100 of ice cold 0.9 % NaCl solution. Part of blood was collected in tubes containing potassium oxalate and sodium fluoride for the estimation of glucose by *O*-toluidine method [17]. Hemoglobin was estimated in heparenized blood [18]. Serum insulin and glucosalated heamoglobin (Hb A1c %) were estimated according to Wilson and Miles [19] and Abraham *et al.* [20], respectively. Serum alanine and aspartate aminotransferase (ALT and AST) and alkaline phosphatase (AP) activity enzymes were estimated according to Reitman and Frankel [21] and Kind and King [22], respectively. In addition, creatinine and urea were estimated according to Bonsens and Taussky [23] and Patton and Crouch [24], respectively. Serum cholesterol, triglycerides, high density lipoprotein cholesterol (HDLc) and low density lipoprotein cholesterol (LDLc) were determined according to Trinder and Ann [25], Young and Pestaner [26], Richmond [27] and Fruchart [28], respectively. Atherogenic index (cholesterol/HDL-c) was calculated according to Castelli and Levitar [29]. In addition, serum glutathione-peroxidase (GPX), superoxide dismutase (SOD), catalase and nitric oxide (NO) were determined by enzymatic colorimetric procedures according to Habig *et al.* [30], Dechatelet *et al.* [31], Sinha [32] and Green *et al.* [33], respectively. Livers samples were analyzed for estimation of glycogen, cholesterol and total lipids according to Rerup and Lundquist [34], Abell *et al.* [35] and Folch *et al.* [36], respectively.

Statistical Analysis: Collected data were presented as mean ±SD and statistically analyzed using one way analysis of variance (ANOVA).Student "t" test was used for significance according to Artimage and Berry [37].

RESULTS

All the treated groups showed significant increase in weight gain and FER and the more improvement in nutritional results appeared in CEV > CE > CPV> AD groups (P<0.01 and 0.001). The lowest values appeared in CP compared with C+. The value of weight gain of CE, CPV and CEV groups was significantly increase compared with AD and CP groups. The value of FER of CEV group was significantly increased compared with CP group as shown in Table 1.

Values of serum ALT and AST were significantly decreased in all treated groups (P<0.01 and 0.001) while serum of ALP and creatinine were significantly decreased in CP, CE, CPV and CEV groups (P<0.05, 0.01 and 0.001) but serum urea was insignificantly difference compared with C+ group. Serum ALT of CP and CE groups was significantly increased while serum AST and creatinine were significantly increased in AD and CP groups compared with CPV and CEV groups as shown in Table 2.

Values of serum glucose and HbA_{IC} were significantly decreased in all treated groups while serum of insulin and HG were significantly increased in all treated groups (P<0.01 and 0.001) compared with C+ group. Serum glucose of AD and CEV groups was significantly increased compared with CP and CE groups. The value of insulin of CP, CE, CPV and CEV groups was significantly decreased compared with AD group. The value of serum HbA_{IC} was significantly increased in CP group compared with AD and CE groups but was significantly decreased in CEV group compared with other groups. There was non-significant difference in HG among the treated groups as shown in Table 3.

Values of serum cholesterol (P<0.05, 0.01 and 0.001), triglyceride, LDLc and cholesterol/ HDLc ratio (P<0.01 and 0.001) were significantly decreased in all treated groups while HDLc (P<0.01) was significantly increased in CP, CE, CPV and CEV groups compared with C+ group. Values of serum cholesterol and triglyceride of AD and CP groups were significantly increased compared with CPV and CEV groups. The value of serum HDLc was significantly decreased but serum LDLc was significantly increased in AD group compared with other treated groups. The value of cholesterol/HDLc ratio of AD group was significantly increased compared with CEV group as shown in Table 4.

Table 1: Mean values ± SD of body weight gain, food intake and FER of the experimental rat groups

Groups variables	(C+)	AD	CP	CE	CPV	CEV
Weight gain (g)	31.77±4.31^{d}	89.11±5.11^{b***}	70.31±6.31^{c**}	92.71±7.81^{a***}	90.31±8.31^{a***}	103.2±10.21^{a***}
Food intake(g/w)	15.99±2.11^{a}	17.3^{l}±2.91^{a}	16.39±2.11^{a}	17.88±2.31^{a}	17.33±3.11^{a}	18.87±3.51^{a}
FER	0.033±0.011^{c}	0.085±0.010^{ab**}	0.071±0.012^{b**}	0.086±0.013^{ab**}	0.086±0.010^{ab**}	0.091±0.014^{a***}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001.

^{abcd}Mean values in each raw having similar letters were not significantly different.

Table 2: The Mean values ± SD of serum ALT, AST and ALP enzymes, creatinine and urea of the experimental rat groups

Groups variables	(C+)	AD	CP	CE	CPV	CEV
ALT(µ /ml)	88.81±8.11^{a}	66.11±7.71^{bc**}	70.31±7.81^{b**}	65.11±7.21^{b**}	59.11±6.31^{c***}	55.14±6.15 ^{c***}
AST(µ /ml)	77.31±7.66^{a}	58.31±7.21^{b**}	57.75±6.41^{b**}	53.11±5.98^{bc**}	49.96±5.78^{c***}	45.81±5.12^{c***}
ALP(µ /ml)	99.61±9.71^{a}	83.51±9.61^{ab}	74.39±8.11^{b**}	70.71±7.96^{b**}	68.51±8.21^{b**}	65.11±8.14^{b**}
Creatinine (mg/dl)	1.87±0.44^{a}	1.57±0.56^{ab}	1.15±0.40^{b*}	0.93±0.11^{bc**}	0.92±0.22^{c**}	0.88±0.23^{c***}
Urea(µ /mg)	45.96±5.84^{a}	47.31±6.48^{a}	41.21±5.21^{a}	40.21±4.91^{ab}	41.11±4.33^{ab}	40.31±5.21^{ab}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001.

^{abcd}Mean values in each raw having similar letters were not significantly different.

Table 3: Mean values ± SD of glucose, insulin, HbA_{IC} and HG of the experimental rat groups

Groups variables	(C+)	AD	CP	CE	CPV	CEV
Glucose (mg/dl)	375.11±33.67^{a}	140.31±14.37^{c***}	173.1±20.71^{b**}	167.3±15.71^{b**}	155.11±13.24^{bc***}	149.31±11.32^{c**}
Insulin (µ/l)	7.89±1.21^{d}	15.96±2.30^{a***}	10.96±2.11^{c**}	11.32±2.03^{b**}	11.99±1.96^{bc**}	13.77±2.14^{ab***}
HbA_{IC}%	6.59±0.77^{a}	4.51±0.60^{c**}	5.11±0.45^{b*}	4.36±0.86^{c**}	4.71±0.46^{bc**}	3.61±0.55^{d***}
HG (g/dl)	9.99±1.20^{b}	13.11±1.31^{a**}	12.71±1.13^{a}	13.69±1.61^{a**}	13.55±1.32^{a**}	14.21±1.40^{a***}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001.

^{abcd}Mean values in each raw having similar letters were not significantly different.

Table 4: The Mean values ± SD of serum cholesterol, triglyceride, LDLc, HDLc and cholesterol/ HDLc ratio of the experimental rat groups

Groups variables	(C+)	AD	CP	CE	CPV	CEV
Cholesterol(mg/g)	187.31±19.31 a	150±9.61 ^{b*}	141.31±11.21 ^{b*}	139.81±10.88 ^{bc**}	128.11±12.21 ^{c***}	117.81±9.61 ^{c***}
Triglyceride(mg/g)	115.77 ±9.21a	87.31±8.61^{b**}	90.11±9.21^{b**}	75.81±7.88^{c***}	79.31±9.61^{c***}	69.41±6.21^{cd***}
LDLc (mg/g)	136.05 ±11.17a	101.96±9.03^{b**}	83.88±8.14^{c***}	84.34±6.96^{c***}	74.44±7.87c^{d***}	62.62±7.81^{d***}
HDLc (mg/g)	28.11±2.31^{b}	31.31±3.21^{b}	39.41±3.41^{a*}*	40.31±4.37^{a**}	37.81±5.11^{a**}	41.31±4.50^{a**}
Cholesterol / HDLc	6.61±1.21^{a}	4.83±0.27^{b**}	3.61±0.60^{bc**}	3.51±0.44^{bc**}	3.41±0.54^{bc**}	2.87±0.33^{c***}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001.

^{abcd}Mean values in each raw having similar letters were not significantly different.

Table 5: The Mean values ± SD of serum GPX, SOD, catalase and NO of the experimental rat groups

Groups variables	(C+)	AD	5% CP	5% CE	CPV	CEV
GPX (μ /mg)	55.11±5.61^{d}	61.31±7.31^{bc*}	77.14±8.31^{b*}	88.31±9.19^{a**}	87.31±8.14^{a**}	91.41±10.31^{a**}
SOD (μ /mg)	18.21±1.41^{d}	23.65±2.71^{c*}	30.21±2.99^{b**}	35.21±3.21^{a***}	31.21±4.11^{ab**}	38.71±4.31^{a***}
Catalase (μ/mg protein)	20.71±1.96^{c}	25.96±3.01^{b*}	27.31±2.71^{ab*}	29.61±2.11^{a**}	31.21±3.21^{a**}	30.14±2.61^{a**}
NO	9.61± 1.03^{a}	6.31±0.66^{b***}	5.17±0.88^{bc***}	5.35±0.71^{c***}	5.11±0.93^{c***}	4.31±0.84^{c***}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001.

^{abcd}Mean values in each raw having similar letters were not significantly different.

Table 6: The Mean values ± SD of some liver cholesterol and total lipids and glycogen of the experimental rat groups

Groups variables	(C+)	AD	CP	CE	CPV	CEV
Cholesterol (mg/g)	8.66±1.51^{a}	5.01±0.33^{b**}	5.01±0.44^{b***}	4.11±0.54^{cd***}	3.86±0.73^{d***}	4.39±0.54^{c***}
Total lipids (mg/g)	45.17±5.11^{a}	37.14±6.41^{b**}	38.87±6.22^{b**}	36.61±7.11^{b**}	38.11±6.31^{b**}	35.21±5.41^{b**}
Glycogen (mg/100g)	4.87±0.33^{d}	8.59±1.03^{ab***}	6.89±1.01^{c**}	7.13±0.99^{bc**}	6.99±0.95^{c**}	9.11±1.10^{a***}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001.

^{abcd}Mean values in each raw having similar letters were not significantly different.

Values of serum GPX, SOD and catalase were significantly increased (P<0.05, 0.01 and 0.001) while NO value (P<0.01) was significantly decreased in all treated groups compared with C+ group. The value of serum GPX of AD and CP groups was significantly decreased compared with CE, CPV and CEV groups but values of serum SOD and catalase were significantly decreased but the value of serum NO was significantly increased in AD group compared with CE, CPV and CEV groups as shown in Table 5.

Values of liver cholesterol and total lipid were significantly decreased (P<0.01 and 0.001) while glycogen value (P<0.01 and 0.001) was significantly increased in all treated groups compared with C+ group. The value of liver cholesterol of AD and CP groups was significantly increased compared with CE, CPV and CEV groups but values of liver glycogen was significantly decreased in CP and CPV compared with AD, CE and CEV groups. There was non significant difference in total lipid among treated groups as shown in Table 6.

DISCUSSION

The improvement of nutritional results was agreed with several studies. Pushparaj *et al.* [6], Kocsis *et al.* [38] and Cani *et al.* [39] reported that chicory contains many essential lipids, vitamins and a variety of sugar and also used for its tonic effect. The root and the leaves of chicory are appetizer, cholagogue, depurative, digestive, diuretic, hypoglycaemic, laxative and tonic. Chicory extract contains inulin and fructooligosaccharides. Inulin behaves like a soluble fiber so increase the viscosity of the stomach content and slow down the rate of gastric emptying of water, nutrients and lipids. Urias-Silvas *et al*. [40] concluded that inulin-type fructans extracted from chicory regulate appetite and lipid/glucose metabolism. It has also promising effects on the body weight and fat mass development. Malabu *et al.* [41] and Kurt *et al.* [42] reported that vanadate treatment significantly reduced food intake and also lowered plasma insulin concentrations. Regional hypothalamic levels of neuropeptide Y, a potent central appetite stimulant that is thought to drive hyperphagia in STZ-induced diabetes. Hypothalamic neuropeptide Y concentrations rise markedly in diabetes and are normalized by insulin replacement. Vanadyl sulfate administration improved the loss in body weight due to STZ-induced diabetes and decreased the rise in blood glucose levels. It is known that chicory contains isoflavones, polyphenols and other antioxidants that can reduce the elevation of serum ALT and AST. Antioxidants in chicory extract have protective activity and improve liver function [3].

In accordance, Sadeghi *et al.* [43] confirmed the hepatoprotective activity effect of the hydroalcholic extract of *Cichorium intybus* as *Cichorium* extract significantly suppressed mainly the increase in plasma activities of AST, ALT and ALP concentration. Shafaq and Tabassum[44] indicated that oral administration of *C. intybus* extract reduced cisplatin-induced nephrotoxicity and also prevented elevated plasma creatinine, urea and nitrate, plasma and tissue MDA levels and restored antioxidant enzymes.Oral administration of vanadium(III, IV, V)-chlorodipicolinate did not alter diabetic serum creatinine and blood urea nitrogen levels, suggesting no significant side effects of vanadium treatment on renal functions at the dose of 0.3 mg/ml in diabetic rats. The anti-diabetic effects of treatment with vanadium complexes were likely associated in part with the oxidation state of vanadium [45].Vanadium has insulinomimetic properties, but also inhibits feeding, which could lower blood glucose. Insulin-mimetic vanadyl-poly complex is proposed as a novel drug delivery system for treating type 1 diabetic animals and results showed improvement in diabetes as seen by results on oral glucose tolerance test, HbA (1c) levels and blood pressure [46].

The lipid parameters results were agreed with Yassin *et al.* [47], who reported that chicory extract improve lipid profiles by lowering plasma total cholesterol and triglyceride concentrations and elevating HDL-c concentration due to presence of inulin. Kim and Shin [48] recorded higher serum concentration of HDL-c and lower serum concentration of LDL-c in rats consumed diets containing 1%, 5% chicory extract or 5% inulin for 4 weeks. Abd El-Ghanny [49] found a significant decrease in cholesterol, LDL-c, urea and creatinine in treated rats groups with vanadium in compared with untreated diabetic rats group. Mukherjee *et al.* [50] recorded that vanadyl sulfate can lower elevated blood glucose, cholesterol and triglycerides in a variety of diabetic models including the STZ diabetic rat, the Zucker fatty rat and the Zucker diabetic fatty rat.

In recent years, the role of free radical damage consequent to oxidative stress is widely discussed in diabetic complications.Chicory supplemented diet is reported to have free radical scavenging and antioxidant properties by restoring glutathione, GSP, SOD and catalase levels. These findings may be due to the presence of antioxidant compounds such as anthocyanins, flavonoids, polyphenols and vitamin C that could contribute to protection against free radicals generation and carcinogenic effects of nitrosamines [8, 51]. Moreover, it was shown that vanadium supplementation to diabetic rats significantly decrease serum antioxidant enzyme levels (superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione-S-transferase) which were significantly raised by diabetes in muscle tissue showing that this trace element could be used as preventive for diabetic complications [42,52]. It is known that diabetes caused hyperglucagonemia and marked decreases in liver glycogen and activities of glycogen-metabolizing enzymes in liver. Moreover, glycogen synthase and the activity ratio (activity of glycogen synthase a divided by activity of total glycogen synthase) decreased to 30% of control levels. Cichorium root extract therapy leads to normalization of some morphofunctional liver features (decreases glycogen content and cell of necrosis and increases the number of cells with pronounced protein synthesis activity) in rats with CCl4-induced hepatitis [5]. Vanadium administration in diabetic rats restored these parameters to near control values and recovered activities of glycogenolytic enzymes (phosphorylase, phosphorylase kinase and protein kinase. So, insulin like *in vivo* action of vanadate on various parameters is related to hepatic glycogen metabolism [53].

It can be concluded that vanadium administered in combination with chicory was the most effective in controlling the altered glucose metabolism and antioxidant status in diabetic rats.

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